Biogot ™ Ni-IDA Sepharose 6B

Background:

Purifying histidine-tagged recombinant proteins by immobilized metal affinity chromatography (IMAC) continues to grow in popularity. Nickel (Ni2+) is the most commonly used metal ion in IMAC purifications. Ni-IDA Sepharose 6B consists of 90 μm beads of highly cross-linked agarose, to which a chelating ligand has been immobilized. This chelating group has then been charged with nickel (Ni2+) ions.

To compare with other Nickel affinity chromatography, Ni-IDA has higher protein-binding capacity. It has low Ni2+ leakage and is compatible with a wide range of additives used in protein purification. Its high flow properties make it excellent for scale-up and gravity flow column purification.

Introduction:

Biogot ™ Ni-IDA sepharose 6B has immobilized more Ni2+ than other Ni-IDA (looks more blue). Our Ni-IDA can couple more protein per ml. Our product is made of GE Sepharose CL gels. The cross-linked form of Sepharose is chemically and physically more resistant than Sepharose itself, offering the same selectivity with better flow characteristics. Cross-linked Sepharose gels are resistant to organic solvents and thus are the choice for separations in organic solvents.

Ni-IDA Sepharose 6B is available in 2, 10, 25 and 100 ml lab packs for different requirement. The medium is stored in 20% ethanol at 4 ℃ to 30 ℃ for longer periods of time.

Table 1. Medium characteristics

Matrix	6% agarose
Particle Size	45 μm-165 μm
pH stability Working Range	3-13
pH stability Cleaning-in-Place (CIP)	2-14
Pressure/Flow Specification	100-200 cm/h, pressure drop cm H2O/bed height=15, bed height 10 cm, column 5 cm i.d.
Flow Velocity	<30 cm/h
Fractionation [Mr] Globular Proteins	1 x 104-4 x 106
Storage Conditions	4 to 30 ℃, 20% Ethanol
Autoclavable	Autoclavable, 20 min at 120 ℃ in pH 7

Recommended buffers:

Native conditions:

Binding/wash buffer: 20 mM Tris-HCl, 500 mM NaCl, 5 mM imidazole, pH 8.0.

Elution buffer: 20 mM Tris-HCl, 500 mM NaCl, 500 mM imidazole, pH 8.0.

Denaturing conditions:

Binding/wash buffer: 20 mM Tris-HCl, 8 M urea, 500 mM NaCl, 5 mM imidazole, pH 8.0.

Elution buffer: 20 mM Tris-HCl, 8 M urea, 500 mM NaCl, 500 mM imidazole, pH 8.0.

Notice: The optimal concentration of imidazole needed in the sample and buffer to obtain the best purity and yield differs from protein to protein. Under native conditions, 20-40 mM imidazole in the binding buffer is suitable for many proteins. 500 mM imidazole in the elution buffer is most often sufficient to completely

elute the target protein.

Sample preparation:

The protocol below has been used successfully in our laboratories, but other established procedures may also work.

1. For protein expressed in E. coli or yeast cytoplasm. Dilute the cell paste: Add 5-10 ml of binding buffer for each gram of cell paste. It is essential that the sample and binding buffers contain the same concenctration of imidazole to prevent binding of host cell proteins with exposed histidines.At the same time , remove large particles and high concentration of reagents such as EDTA, amino acids and reducing agents, which can destroy Ni-IDA resins.

2. Enzymatic lysis: 0.2 mg/ml lysozyme, 20 μg/ml DNAse, 1 mM MgCl2, 1 mM PMSF (final concentrations) or other protease inhibitors. The inhibitors must have no effect on the ability of the Ni resin.then Stir for 30 minutes at +4 ℃.

3. Mechanical lysis: Sonication, homogenization, repeated freeze/thaw or similar techniques.

4. Adjust the pH of the lysate to pH 7.4: Do not use strong bases or acids for pH-adjustment (precipitation risk).

5. Centrifuge the lysate: Transfer to tubes and centrifuge at 12000rpm for 20 minutes at room temperature or +4 ℃ depending on the sensitivity of the protein.

6. Collect supernatants and perform the purification.

7. For proteins secreted into culture medium by yeast, insect, or mammalian expression systems,if have a lot of large volume of supernatant, concentrate the proteins by ammonium sulphate precipitation Dialyze with 1 × PBS,then apply to the column, If bit culture supernatant does not contain EDTA, histidine, or any other reducing agents that might affect the Ni column, it can be applied directly to the column.

Note: You can also apply unclarified sample to the column (i.e. omitting step 5 above). If so, extend the mechanical lysis somewhat, e.g. sonicate for 10 minutes. Total purification time will increase due to the higher viscosity of the unclarified sample.

Purification Procedures:

1. Column preparation.

(a) Mix the slurry by gently inverting the bottle several times to completely suspend the resin.

(b) Use a pipette to transfer an appropriate volume of Ni resin slurry to the column. Allow the resin to settle and the storage buffer to drain from the column.

(c) Equilibrate the column with four bed volumes of binding/wash buffer or until A280 is stable.

2. Purification protein

(a) Binding the protein to the resin. Apply the above soluble cleared sample containing His-tagged protein to the column with a flow-rate of 0.5-1 ml per minute. Collect and save the flow-through for analysis.

(b) Washing. Wash the column with eight bed volumes of binding/wash buffer or until A280 is stable at the flow-rate of 1 ml per minute.

(c) Elution of the target protein. Elute the polyhistidine-tagged protein with five to ten bed volumes of Elution buffer. Collect the elute and dialyze it against 20 mM Tris-HCl pH 8.0 or 1 ×PBS, pH 7.4, according to the specific application of the target protein.

3. Purification of polyhistidine-tagged proteins (expressed mainly in inclusion bodies) from E. coli under

Denaturing conditions:

(a) Resuspend the cell pellet in 1 × PBS (about 5 ml per ml of pellet), and disrupt cells by sonication as described above.

(b) Collect inclusion bodies by centrifuging the lysate at 12,000 rpm for 10 minutes. Wash inclusion bodies with 1×PBS several times if necessary.

(c) Solubilize the inclusion bodies in binding/wash buffer (about 5 ml per ml of pellet), and incubate for 30-60 minutes at room temperature. Homogenization or sonication may be necessary to fully solubilize the pellet.

(d) Centrifuge at 12,000 rpm for 30 minutes to remove any remaining insoluble material. Carefully transfer supernatant to a clean tube without disturbing the pellet and load it on the Ni column pre-equilibrated with binding/wash buffer.

(e) Wash the column with binding/wash buffer for 3-4 times until the absorption at 280 nm is close to zero.

(f) Elute with minimal volume of elution buffer.

Note: The process recommended here is the purification of protein from inclusion body, the eluted protein from this process may need to be refolded to obtain the active and soluble protein.

Regenerating the medium

Note: The medium does not have to be stripped between each purification if the same protein is going to be purified; it is sufficient to recharge the medium after 5–7 purifications, depending on the cell extract volume, target protein, etc.

To recharge Ni-IDA Sepharose 6B, first remove residual Ni2+, wash with 5 column volumes 20 mM sodium phosphate, 0.5 M NaCl, 50 mM EDTA, pH 7.4.

Remove residual EDTA by washing with at least 5 column volumes of binding buffer followed by 5 column volumes of distilled water before recharging the column.

To recharge the water-washed column, load 0.5 column volumes of 0.1 M Ni2SO4 in distilled water. Salts of other metals, chlorides or sulfates, may also be used.

Wash with 5 column volumes of distilled water followed by 5 column volumes of binding buffer (to adjust pH) before storage in 20% ethanol.

Ordering information

Product	Quantity	Code No.
Ni-IDA Sepharose 6B	2 ml	BD0052-1
Ni-IDA Sepharose 6B	10 ml	BD0052-2
Ni-IDA Sepharose 6B	25 ml	BD0052-3
Ni-IDA Sepharose 6B	100 ml	BD0052-4

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